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2016 TUA MID-YEAR MEETING

Podium

Podium-1
Oncology

PD1-1:

REST MODULATES HYPOXIA-INDUCED NEUROENDOCRINE DIFFERENTIATION OF PROSTATE CANCER CELLS BY ACTIVATING AUTOPHAGY SIGNALING

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Purpose: To study the role of element-1 silencing transcription factor(REST) in hypoxia induced neuroendocrine differentiation(NED) of prostate cancer.
Materials and Methods: Prostate cancer cell line LNCaP was cultured in hypoxic chamber to induce NED. Neuroendocrine differentiation was quantified by neurite length measurement by phase-contrast optical microscope. Inducible REST knock down and overexpression LNCaP cell line was established and used to determine the effect of REST in NED in LNCaP cells. RNA was harvested from hypoxia-treated LNCaP, normoxia LNCaP, induced REST knock down LNCaP cells and sequenced with Illumina Genome Analyzer*II*.
Results: REST, a transcriptional repressor of neuronal genes that has been implicated in androgen-deprivation and IL-6 induced NED, is essential for hypoxia-induced NED of PCa cells. Bioinformatics analysis of transcriptome profile of REST knockdown with hypoxia treatment demonstrated that REST is a master regulator of hypoxia-induced genes. Gene set enrichment analysis (GSEA) of hypoxia and REST knockdown co-upregulated genes revealed their correlation with HRPC. Consistently, gene ontology (GO) analysis showed that REST reduction potential associated with hypoxia-induced tumorigenesis, NE development, and AMPK pathway activation.
Conclusion: REST knockdown alone is capable of activating AMPK and autophagy activation is essential for hypoxia-induced NED of PCa cells.

PD1-2:

INHIBITION OF CISPLATIN-INDUCED AUTOPHAGY ENHANCES APOPTOTIC CELL DEATH IN HUMAN BLADDER CANCER CELLS

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Purpose: Bladder cancer (BC) is a common urologic cancer with high recurrence rate. Cisplatin is the first member of a class of platinum-containing anti-cancer drugs that binding to and causing DNA cross-linking which ultimately leads to apoptosis. Cisplatin is used to treat various types of cancers including BC. However, cisplatin alone is not very effective in BC and the combinations of gemcitabine/cisplatin is now the first-line chemotherapy for muscle invasive BC. Our previous studies showed that BC cells exhibits high basal level of autophagy and treatment of chemotherapeutic agents further induces autophagy as a survival mechanism. In this study, we investigated if cisplatin induces autophagy in human BC cells and whether inhibition of cisplatin-induced autophagy enhances cancer cell death.
Materials and Methods: The cell viability of RT4 (grade I), 5637 (grade II), and T24 (grade III) human bladder cancer cells treated with cisplatin alone or combined with autophagy inhibitor, bafilomycin A1 (BafA1), was accessed by WST-8 cell viability kit. The autophagy status in cells was performed by the detection of microtubule-associated light chain 3 form II (LC3-II) using immunofluorescent staining and Western blot. Moreover, the formation of autophagolysosome was detected using transmission electron microscopy to confirm the induction of autophagy in cisplatin-treated T24 cells. To investigate the signaling pathway involved in cisplatin-induced autophagy, the activation of AKT, ERK, AMPK and MAPK and the inhibition of mTOR in cisplatin-treated cells were detected using Western blot. Induced apoptosis was determined by the detection of cleavage caspase 3, and the measurement of caspase 3/7 activity and DNA fragmentation in treated-cells.
Results: Advanced bladder cancer cells (5637 and T24) were more resistant to cisplatin than RT4. The processing of LC3-II was elevated in cells treated with increased concentration of cisplatin, suggesting cisplatin induces autophagy. Detection of autophagy flux by blocking autophgosome to lysosomes fusion using Baf A1 and the direct observation of autophagolysosome formation in cisplatin-treated T24 cells using TEM further confirmed that cisplatin indeed triggers autophagy in BC cells. Activation of AKT, ERK and MAPK signaling and inhibition of mTOR was detected in cisplatin treated cells. However, pretreatment of specific inhibitors of ERK, MAPK did not attenuated cisplatin-induced autophagy suggests these pathways are not involved in the induction of autophagy. Finally, reduced cell viability and induced apoptosis were observed in cisplatin-treated cells pretreated with autophagy inhibitor suggesting that inhibition of autophagy enhances cancer killing effect of cisplatin in human BC cells.
Conclusion: Cisplatin induces autophagy through activation of AKT and inhibition of mTOR in human BC cells. Our data suggest that autophagy inhibition promotes apoptosis in cisplatin-treated cells, and could be a new therapeutic paradigm for the treatment of bladder cancer.

PD1-3:

NOVEL BLADDER CANCER BIOMARKERS DISCOVERY BY MICRODISSECTED COMPARATIVE TISSUE PROTEOMICS

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Purpose: More than 380,000 new cases of bladder cancer are diagnosed worldwide, accounting for 150,200 deaths each year. No reliable biomarker for bladder cancer is used clinically. We employed a strategy combining laser microdissection, isobaric tags for relative and absolute quantitation labeling, and liquid chromatography-tandem MS (LC-MS/MS) analysis to profile proteomic changes in fresh-frozen bladder tumor specimens.

Materials and Methods: Cellular proteins from four pairs of surgically resected primary bladder cancer tumor and adjacent nontumorous tissue were extracted for two batches of isobaric tags for relative and absolute quantitation (iTRAQ) experiments. The DAVID (database for annotation, visualization and integrated discovery) is used for analysis of dysregulated proteins. ELISA were employed for validation of candidate tumor markers in tissues and the age-matched urine specimens from bladder cancer patients and hernia patients as control were compared.
Results: The iTRAQ experiments identified a total of 3220 proteins. Seven differentially expressed proteins were selected as potential bladder cancer biomarkers for further verification. Immunohistochemical analyses showed significantly elevated levels of three proteins- SLC3A2, STMN1, and TAGLN2- in tumor cells compared with noncancerous bladder epithelial cells, and suggested that TAGLN2 could be a useful tumor tissue marker for diagnosis (AUC = 0.999). The DAVID analysis revealed three top-ranking biological processes as involved in extracellular matrix organization, extracellular structure organization, and oxidation-reduction. ELISA revealed significantly increased urinary levels of both STMN1 and TAGLN2 in bladder cancer subgroups compared with control groups. Urinary TAGLN2 in bladder cancer samples showed the largest fold change (7.13-fold), with an AUC value of 0.70 ($p < 0.001$, n = 205).
Conclusion: Our study discovered that TAGLN2 showed the most significant over expression in bladder cancer tissues and urine specimens, and thus represents a potential biomarker for noninvasive screening for bladder cancer.

PD1-4:
ANALYSIS OF PROSTATE CANCER FOCI IN PATIENTS WITH TRANSRECTAL 10-CORE SYSTEMIC RANDOM BIOPSY—CAN WE PREDICT PATHOLOGICAL STAGE OF PROSTATE CANCER THROUGH TRUS BIOPSY?

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Purpose: The aim of this study is evaluating the detection site of prostate cancer foci in patients receiving transrectal ultrasound(TRUS) guided 10-core systemic random biopsy and comparing the relationship between digital rectal examination and prostatic biopsy. The study ultimately aims to predict pathological staging of prostate cancer by TRUS-biopsy.
Materials and Methods: Between September 2005 and June 2014, 1314 men received TRUS-guided biopsy were included in this study. Indications for TRUS guided prostate biopsy were: abnormal digital rectal examination and/or a serum PSA over 4.0 ng/ml. 701 patients were excluded due to less than 10 cores or received target biopsy and 68 patients were excluded due to different machine. Finally, 545 patients underwent at least 10-core random biopsy protocol by TRUS and 46 of those patients treated with radical retropubic prostatectomy.
Results: Of the 545 patients, one hundred and fifty-two (27.9%) were positive for prostate cancer, including 64 of 370 (16.2 %) men with normal DRE and 88 of 175 (50.3 %) with abnormal DRE. Patients with normal DRE exhibited younger age (67.9 vs. 71.0 yrs, p = 0.007), lower serum PSA(15.7 vs. 25.6 ng/ml, p = 0.0032), lower PSA density(0.41 vs. 0.71, p = 0.0016), less positive cores(p = 0.022), and less Gleason score(p = 0.0006) than those with abnormal DRE.
46 of those patients treated with radical retropubic prostatectomy, including 17 men with normal DRE and 29 with abnormal DRE. The pathological finding revealed higher number of positive biopsy cores in advanced staging whether digital finding was positive or not. For evaluating the association of the detection site of prostate cancer foci and pathological staging, we defined low, intermittent, and high risk group according to number of positive cores in different locations (lateral, parasagittal, and apical area). We identified low risk group with no positive core. Intermittent risk group means one positive core or 2 positive cores ipsilateral (2S). High risk group means 2 positive cores contralateral (2C) or number of positive cores more than 3. Otherwise, we also reclassified pathological staging into 3 subgroups: pT2a and pT2b, pT2c, and pT3a at least. The pathological finding of patient with low risk group will below pT2c and high-risk group will above pT3. ($p = 0.031$ in lateral sites, $p = 0.007$ in parasagittal sites, and $p = 0.023$ in apical sites).
Conclusions: In our study, patient with normal digital rectal examination and PSA elevation received TURS-biopsy has 16% malignancy change. Besides, patients with abnormal digital rectal examination received TURS-biopsy has 50% malignancy change. Patients with normal DRE exhibited younger age, lower serum PSA, lower PSA density, less positive cores, and less Gleason score than those with abnormal DRE. The cancer foci detected by biopsies of the prostate are equally distributed even subgroup analysis depending on location of positive cores or prostate size. Prediction of pathological stage in prostate cancer through TRUS 10-core random biopsy is available in this study.

PD1-5:
DOWNREGULATION OF MIR-145 PREDICTS A WORSE OUTCOME IN UPPER TRACT UROTHELIAL CARCINOMAS

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Purpose: MicroRNAs (miRNAs) represent a class of small non-coding RNAs regulating gene expression by inducing the degradation of RNA or interfering with translation. Aberrant miRNA expression has been described in several types of cancer in humans. By our pre-set miR array data, we identified miR-145 as the most significant tumor suppressor mir in urothelial carcinoma (UC). However, the expression of miR-145 in UC of the upper urinary tract (UTUC) has not been investigated. This study is conducted to evaluate the outcome predictive value of miR-145 expression in UTUC.
Materials and Methods: Using a miRNA array (Applied Biosystems) that included 667 human miRNAs and mammalian RNU6B, we compare the differentially expressed miRNAs between BFTC909 cell line and paired UTUC samples. The miR-145 expression levels from 65 UTUC tissues and the paired adjacent noncancerous tissues were investigated by Real-Time Reverse Transcriptase PCR assay. In addition, the functional consequences of miR145 transfection in BFTC909 cells were studied *in vitro* by MTT, wound healing, cell migration, cell invasion assays.
Results: In 65 paired tissues, we found that mir-145 expression was significantly decreased in UTUC tissues than in paired adjacent noncancerous tissues. Decreased mir-145 expression was associated with worse recurrence-free and cancer-specific survival. In cell line experiments, we demonstrated that mir145 can inhibit cell proliferation, migration and invasion in BFTC909 cell line after transfection of miR145 mimics.
Conclusion: Our findings imply that decreased miR-145 expression is a potential biomarker to predict clinical outcome of UTUC patients. Further study is necessary to identify the molecular mechanisms of miR-145 involved in the cancerous processes of UTUC.

PD1-6:
BY INHIBITING SNAIL SIGNALING, OSTHOLE SUPPRESSES THE EMT-MEDIATED METASTATIC ABILITY IN PROSTATE CANCER

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Purpose: To evaluate the anti-metastatic potential of osthole in prostate cancer cells and xenograft animal model.
Materials and Methods: The PC3 and DU145 human adrogen independent prostate cancer (AIPC) cell lines were obtained from American Type Culture Collection for cell assay. PC-3M, a highly metastatic subline derived from the hepatic metastasis of PC-3 in nude mice, was used for xenograft